It appears to me not improbable that the comparative examination of a sufficient series of specimens would demonstrate that this species and *M. elegans* are founded on half-grown specimens.

Iphis septemspinosa (Fabricius).

Celebes, Macassar (a female).

Arcania novemspinosa (White), var. *aspera*, n.

A specimen (adult female) without definite locality differs from White's type of *novemspinosa* in the British Museum in the broader and much more closely granulated carapace, and the relatively shorter spines of the posterior and posterolateral margins. White's specimen is a male. Although the characters distinguishing the genera *Iphis* and *Arcania* are scarcely of generic value, it may be convenient to retain the former name for the Fabrician *septemspinosa*, to which it has long been applied, and which differs somewhat more markedly from the species of *Arcania* than these do among themselves.

Dorippe sima, M.-Edw.

Borneo (an adult female).

[To be continued.]

XXXI.—*On Hypochlorin and the Conditions of its Production in the Plant.* By Prof. PRINGSHEIM*.

In a previous communication† I called attention to the existence in green vegetable cells of a body to which I gave the name of "hypochlorin," on account of its close relationship to chlorophyll. I now give some more detailed statements as to its occurrence and microchemical characters, and append thereto some further remarks upon the constitution of the chlorophyll-bodies.

So far as they regard hypochlorin, these statements relate essentially to the behaviour of this body at high temperatures and to the conditions of its production in the seedling plant. With regard to the chlorophyll-bodies, they will at the same

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† Monatsb. Berl. Akad. July 1879; translated in this Journal for January 1880.

time indicate a noteworthy structure of those bodies which has hitherto not been noticed by anatomists, and demonstrate the wide diffusion of fatty oil in them.

I. *Structure and Composition of the Chlorophyll-bodies.*

In the direct observation of the fresh plant the hypochlorin cannot be distinguished in the apparently homogeneous chlorophyll-bodies; for it is only in rare instances that traces of its presence can be detected in them under normal conditions. Its presence betrays itself at once, however, when the green cells are treated with hydrochloric acid.

Under the influence of this acid, as I have already briefly stated in my former memoir, dark, deep-reddish-brown or rust-coloured, irregularly bounded forms are separated in a few hours in the chlorophyll-bodies, especially at their periphery, and also between neighbouring chlorophyll-bodies. These are not perfectly spherical drops, but rather semifluid masses of irregular form, with sometimes spherical, sometimes plane limiting surfaces, which sometimes push forth angular or pointed processes, and thus become indistinct crystalline scales or nests. From these, after a longer or shorter time, shoot forth long, pointed, straight or curved needles, and extremely thin twisted filaments, or even shorter and thicker rods.

These extremely remarkable effects, which occur without exception in all chlorophyll-plants after treatment with hydrochloric acid, constitute a characteristic and infallible reaction for hypochlorin. Without any further examination, they demonstrate directly by their external appearance the presence of a hitherto unnoticed substance in the chlorophyll-bodies of plants. But the circumstances under which they occur present many difficulties in the way of the right conception of the relation here brought to light, and therefore require a more thoroughgoing examination and investigation.

As has already been stated, even direct observation leaves no doubt that the substance which afterwards (perhaps modified by the process of its production) becomes converted into the crystalline needles outside the chlorophyll-bodies was previously present in their fundamental substance. It is evidently drops of an oleaginous consistency which first separate from the fundamental substance, gradually increase or coalesce into larger masses, and form the foundation of the future needles and filaments. No distinct structure is recognizable in these needles and filaments; and one may often be in doubt, when the formations are thicker and shorter, whether they possess an organic structure or a crystalline texture.

Of all known histological formations they remind us most (and, again, especially the thicker and twisted forms) of the bacillar forms of many so-called wax coatings of leaves, which, as is well known, consist of diverse mixtures of substances poor in oxygen. And as from their conditions of occurrence and solubility they are evidently organic formations which appear to belong to a group of proximate constituents of the plant allied to these wax bacilli, and also visibly proceed from a common oleaginous parent substance, one may easily suppose that they consist of a mixture of resin and essential oil, such as occurs not unfrequently in vegetable tissues. The consistency, the limitation, the solubility, and difficult mobility of the separating drops more nearly resemble essential than fatty oils.

By the absence of the pure drop-form, and still more by the directly recognizable processes of conversion of the surface into multifarious structures of indistinctly crystalline texture, these imperfectly fluid products of separation produce of themselves the impression of a mixture of solid and fluid substances, or rather of a kind of mother liquor of a separating solid compound, whilst, at the same time, they give rise to the idea of an essential oil in process of resinization. Further, this microscopical character agrees with the behaviour to all known solvents of resins and oils.

All the forms under which these segregations make their appearance, the irregularly limited drops, the crystalline scales, needles, filaments, &c., are insoluble in water, in saline solutions, and in dilute mineral and organic acids; but they dissolve readily and completely in ether, benzole, sulphide of carbon, and essential oils, and also in absolute or even in moderately dilute alcohol, although frequently only after a considerable time, and with more or less difficulty.

The constituents of which this hypochlorin mixture consists have not previously been distinguished in the fundamental substance of the chlorophyll-bodies, with the exception of the colouring-matter which they contain. Nothing especially has ever yet been known of a body with the properties of hypochlorin and its peculiar forms. The deep coloration, however, of the drops and needles might lead many to suspect (as I have found during the demonstration of these formations) that the separated drops in their whole mass consist only of the colouring-matter of the chlorophyll, which, being separated from the fundamental substance by the hydrochloric acid, becomes solidified or crystallizes in the form of needles and filaments.

But this is not the case. That the colour of the separated

masses is due to the colouring-matter of the chlorophyll is undoubtedly correct: but this colouring-matter does not form any fluid of itself; and as it is insoluble in dilute and even in concentrated hydrochloric acid, as is shown by direct observation if we treat the chlorophyll-bodies in the unopened cell with hydrochloric acid, a special menstruum must exist in the separated masses, which serves as the bearer of the colouring-matter that tinges the drops and needles. This, indeed, becomes directly perceptible; for the needles, filaments, and rods often *lose their colour* when they have become older and been for a considerable time exposed to the light, completely retaining their form, however; and in many cases the rigid structures are *colourless even at their production.* Their colour is therefore due solely to a contamination with colouring-matter which has been carried over.

The drops separating under the action of hydrochloric acid consist, therefore, of an oleaginous fluid tinged with dissolved chlorophyll-colouring-matter, which is either itself crystallizable or contains a crystallizable substance, "hypochlorin;" and therefore, as will be seen from this statement, I understand under the name "hypochlorin" not the entire mixture of which the masses separable by hydrochloric acid from the chlorophyll-bodies consist (which, indeed, includes the colouring-matter tinging them), but only the body contained in them which afterwards solidifies in an indistinctly crystalline form, or (which is the same thing) the foundation of this crystallizable body originally present in the chlorophyll-grain. For it may still appear questionable whether the body which subsequently acquires a crystalline texture outside the chlorophyll-body was present with the same properties in the fundamental substance, or undergoes, during its passage out, a change which causes its solidification and crystallization.

Moreover, I will remark, we may regard the hypochlorin reaction, *i.e.* the formation of the dark secretions from the chlorophyll-bodies, as not a specific action of hydrochloric acid; for it is produced also by other agents.

Picronitric acid, for example, in various degrees of dilution, produces in most cases precisely the same effect as hydrochloric acid, but does not furnish such clear images, as it attacks the fundamental substance of the chlorophyll-bodies more strongly, by which means the forms become more indistinct.

In all preparations of green tissues which have lain for months or years in glycerine or chloride of calcium, the dark indefinitely bounded hypochlorin-masses also appear here

and there, separated spontaneously, as it were, from the chlorophyll-bodies.

It is therefore certain, even from our present experience, that the oleaginous substance which is a constituent of every chlorophyll-grain can be separated therefrom by various means. It would almost appear that this can be effected purely mechanically by displacement and disturbed adhesion.

One of the simplest means of separation is the application of moist heat. If green tissues be heated with water, or distilled with aqueous vapour, drops of an oleaginous substance separate from the fundamental substance of the chlorophyll-bodies. The phenomenon is analogous to that which Briosi produced in the chlorophyll-bodies of the Musaceæ by treatment with cold water. In his fine memoir on this subject * he assumes (and in this later observers have followed him) that the normal occurrence of oil in the chlorophyll-bodies of the Musaceæ is an exceptional case, forming as it were a substitute for deficient starch. But, as I shall here show, the occurrence of oil in the chlorophyll-bodies of plants is quite a general phenomenon and certainly not directly dependent on the presence or absence of starch-enclosures; only, it would appear, the escape of the oil from the chlorophyll-bodies does not take place in most plants except by treatment with water of higher temperature.

In many plants a heat of 50° C. (=122° F.) suffices; and at this temperature any starch-enclosures that may be present do not swell up. Other plants require higher temperatures, when, in consequence of the swelling of the starch-enclosures or the bursting of the whole chlorophyll-grain, the comprehension of the process may be rendered more difficult by collateral circumstances. The phenomenon is also easily called forth if the tissues are exposed for from ten to fifteen minutes or longer to the vapours of boiling water.

In all cases there are, after this treatment, as already indicated, at the periphery of the chlorophyll-bodies, some smaller or larger oil-drops, which clearly have issued from the chlorophyll-bodies under the action of the warm water or of the hot vapour.

The chlorophyll-bodies themselves at the same time acquire the nature of hollow bodies, which may put on a different appearance in different plants according to the temperature employed and the duration of the action. They either constitute spongy porous masses, or form hollow trabecular networks, or, lastly (especially when strong swelling with

* Bot. Zeitung, 1873, p. 529.

bursting of the whole grain has taken place), they consist only of the torn fragments of the envelope of the ruptured grain. All these solid residues are formed of the so-called protoplasmatic foundation of the chlorophyll-body and its inflated starch-enclosures, and are more or less strongly tinged with green by nearly unaltered chlorophyll-colouring-matter.

The oil-drops separated from these solid residues by the warm water or hot vapour, and escaped from the chlorophyll-bodies, *which always dissolve readily and completely in alcohol or ether*, are also tinged more or less with chlorophyll-colouring-matter, most of them in different tints of green and blue; but the darker ones even appear reddish brown, and then, leaving out of consideration the smaller size, produce the same external impression as the first-mentioned drops of the hypochlorin-mixture separated by hydrochloric acid. Nevertheless I do not think that they are identical with the latter. They are distinguished not only by the less degree and generally greater purity of their chlorophyll-green coloration, but also by their readier solubility in alcohol, and, lastly, by their more regular drop-like shape and especially by their permanence in heat. I therefore (as I may here state in anticipation) hold that these oil-drops represent a second non-volatile and uncrystallizable oil present in the chlorophyll-body, which exists in it side by side with the volatile and crystallizable hypochlorin, and in association with the latter forms those irregular masses which issue from the chlorophyll-bodies under the influence of hydrochloric acid.

My reasons for this opinion are as follows:—

It is, in the first place, exceedingly striking that the oil-drops extractible from the chlorophyll-bodies by heat are very much inferior in their mass to the masses of oleaginous substance which can be separated from the same chlorophyll-bodies by hydrochloric acid. Of those large, irregularly bounded, diversely pointed and angular masses which appear under the influence of hydrochloric acid, nothing is to be seen under the action of moist heat. The drops which issue in this case are smaller and more or less exactly spherical; and they do not solidify, but remain fluid. They contain none, or mere traces, of that crystallizable substance which, in the masses separated by hydrochloric acid, calls forth those singular changes of form which I have already described.

We cannot, however, assume that this substance is still present in the solid residues of the heated chlorophyll-bodies and was merely not separated by the action of heat; for by subsequent treatment with hydrochloric acid no further increased or fresh separation of oil can be effected. With

respect to the oil separated, hydrochloric acid calls forth no further change in the behaviour of the chlorophyll-bodies; and the great difference herein shown in the action of hydrochloric acid upon chlorophyll-bodies, according as the latter have or have not previously been heated, appears the more noteworthy, as the chlorophyll-colouring-matter in itself undergoes no essential alteration by heating, especially when the application of heat is of short duration and the temperatures are not high. For the modification that chlorophyll undergoes spectroscopically when heated in water appears to be chemically of no great consequence; so that, as is well known, for many reasons it might even be advisable, in order to render the solution of chlorophyll more persistent, to boil the green tissues with water before the extraction.

The fact remains established:—The remarkable reaction that hydrochloric acid produces in *unheated* chlorophyll-bodies does not occur in those *which have been heated*; and the cause of its non-occurrence cannot be sought in any alteration of the *colouring-matter*.

As the interruption of the hydrochloric-acid reaction for hypochlorin by previous heating of the tissues is fitted to give us a closer insight into the properties of that body, it may be here specially elucidated by a few examples.

If filaments of *Cladophora* in the fresh state are treated directly with hydrochloric acid, the larger hypochlorin-masses (already repeatedly described) make their appearance in every cell without exception. In strongly vegetating filaments with abundant cell-contents, the latter are, as it were, overcrowded with the masses formed by the hypochlorin-mixture. But if the filaments of *Cladophora* are previously heated only from five minutes to a quarter of an hour in water of 50° C. (=122° F.), under which treatment the cell-contents remain essentially unaltered in colour and form, and even appear more transparent than before, and starch-grains and amylum-foci do not swell up, nothing of the hypochlorin-mixture is to be detected in them after the same treatment with hydrochloric acid. All those numerous larger drops, scales, &c. which the hydrochloric acid brings forth in the fresh are wanting in the heated filaments.

A similar behaviour is presented by filaments of *Œdogonium*, *Mesocarpus*, and *Spirogyra*, and, in general, in Algæ with so-called amorphous chlorophyll. In these a still shorter exposure to heat and a lower temperature will often suffice.

Even in the well-limited chlorophyll-bodies of the *Nitellæ* and *Charæ*, and many higher plants with delicate leaves, *e. g.*

in *Elodia*, *Callitriche*, &c., the heating of the whole plant in water of 50° C. for from a quarter to half an hour is sufficient for the complete suppression of the hypochlorin reaction. Other plants require that the action should be of longer duration or the temperature higher. A brief boiling of the tissue in water or treatment of the plant with the vapour of boiling water leads, however, to the same result in all of them.

After such treatment as has been stated, the hypochlorin reaction with hydrochloric acid no longer makes its appearance in the tissues, or at any rate not to the same extent as in the fresh tissues.

There is especially a regular absence of all those larger crystalline scales which the hypochlorin-mixture produces in the fresh plant under the influence of hydrochloric acid. In the tissues heated to a considerable temperature with water, or boiled, or subjected to distillation with water (even when they are subsequently treated with hydrochloric acid) there are now at the periphery of the chlorophyll-bodies only those few and isolated small oil-drops which, as I have already described, separate from the fundamental substance by the action of heat alone, and which, without undergoing any further alteration by hydrochloric acid, obstinately retain the fluid state even under a continued application of heat.

It consequently appears the simplest course to refer the interruption of the hypochlorin reaction by heat, and the non-appearance of the crystallizable segregations when the green tissues are merely heated, to the fact that the peculiar matter in the hypochlorin-mixture which causes its crystalline solidification is destroyed in the chlorophyll-bodies or dissolved by warm water, or becomes volatilized with the hot aqueous vapours.

The latter is my opinion. This microscopic behaviour of the chlorophyll-bodies when heated and the above conception are in agreement with certain attempts which, under the supposition that hypochlorin is a volatile substance, I have made with the view of preparing it on the large scale for chemical analysis, by the distillation of green tissues with superheated steam.

In this way, in fact, we may obtain from the green tissues of very different plants (even of such as possess in the tissues in question no known specific essential oil) a small quantity of a homogeneous essential oil, which separates from its solution in ether in colourless microscropic crystals, assuming the form of small, *curved*, isolated, dendritically-branched needles, which are remarkably similar to the hypochlorin-needles such as separate under microchemical treatment from the hy-

pochlorin-mixture in the cells. The agreement is especially striking in those cases in which the hypochlorin-needles occur in the interior of the cells *isolated* and *perfectly colourless*, or form *small dendritic aggregates*.

I must, however, report hereafter upon these experiments in distillation on the large scale and the products obtained in them; I chiefly refer to them here only for the purpose of indicating the probable connexion of their results with the changes which the chlorophyll-bodies undergo anatomically when they have been heated in water or exposed to hot aqueous vapour.

In favour of the assumption that hypochlorin is a volatile substance, and that a second non-volatile oil is present with it in the hypochlorin-mixture that may be prepared by hydrochloric acid, we have further the behaviour under heat of the *formed* hypochlorin-masses. Thus when green vegetable tissues, in which the hypochlorin-mixture has been separated by hydrochloric acid, and in which it has already acquired the forms of crystalline masses, scales, or nests, are *subsequently* boiled with water, or exposed to aqueous vapour, these segregations gradually lose their crystalline character and, if the action be continued long enough, become converted into clearly spherical oil-drops, which are then unalterable and persistent in heat, and, instead of the previous rust-coloured tint of the hypochlorin-masses, acquire more or less of a chlorophyll-green colour, becoming changed first into olive-green and then to bluish- or grass-green drops. But if long needles and filaments have already separated from the hypochlorin-mixture, the volatilization appears to be more difficult, although even these forms are attacked by the hot aqueous vapours if the distillation be continued for a considerable time.

From the anatomical facts here stated, therefore, the composition of the chlorophyll-bodies is more complex than it appeared to be from previous representations. The existence of oil in them is no exceptional case (here a substitute for deficient starch) confined to a few plants, or, as some people would have it, a pathological condition; but it is *generally diffused* and in essential connexion with the function of the chlorophyll-bodies. At the same time, the hypochlorin is contained in this oil—that colourless volatile substance, crystallizable on separation from the chlorophyll-bodies, which is present as a constant associate of chlorophyll in all chlorophyll-bodies which have been produced in the light.

Further, the phenomena which accompany the separation of the oil from the fundamental substance also furnish us with

information as to the *intimate structure* of the chlorophyll-grain, hitherto not noticed by anatomists, and as to the *local distribution* of the oil among the solid constituents of its fundamental substance.

With the exception of some still but imperfectly investigated cases (such, for example, as the generally known one of *Bryopis*), and leaving out of consideration the sporadic or temporary occurrence of isolated and limited starch-inclusions, the chlorophyll-bodies of the uninjured cell usually produce the impression of homogeneous bodies, apparently consisting of homogeneous green substance. But when the hypochlorin and the oil are extracted from them by evaporation and hydrochloric acid, they are found to be *hollow bodies*, the cavities of which are filled with oil.

The framework of the solid substance exhibited by the chlorophyll-bodies when deprived of oil may, indeed, show subordinate modifications in its forms, according to the species of plant and the age of the tissue, especially when its shape has been influenced at high temperatures by the swelling of starch-enclosures; but, at the same time, the general structure of the chlorophyll-grain is always unmistakably manifested, as that of *a porous body*, *in the pores of which the oil has accumulated.* Its solid residues, if the grain has not been broken up into separate shell-like fragments by complete disruption, always represent more or less distinct and often exceedingly elegant *hollow bodies with perforated envelopes*, which latter may assume all possible forms of a retiform trabecular framework. These forms show themselves most distinctly, perfectly regular, characteristic, and always homogeneous when the extreme action of heat is avoided, and the process of removal of the oil is carefully conducted.

The right mode of proceeding must here be specially ascertained for each case, as it is influenced by the actual condition of development of the chlorophyll-body, and especially by the grade of development of its starch-enclosures. It is, however, always easily attainable, if the temperature and the duration of its action are suited to the given conditions. The tissues must be, according to their constitution, exposed for from a quarter to half an hour to the vapour of boiling water, or heated for about half an hour in water of 50°–80° C. (=122°–176° F.), and then left for at least one or two days lying in dilute hydrochloric acid. A good strength of the acid solution is one volume of hydrochloric acid to four volumes of water. The tissues may, however, remain for weeks or even months in the dilute hydrochloric acid without any alteration; and the structure of the chlorophyll-bodies thus gains, or at

any rate does not lose, in sharpness. If the chlorophyll-bodies contain but little starch or none at all, the tissues may without injury or even with advantage be boiled for a short time (about half an hour) in water before their treatment with hydrochloric acid. In many cases the reverse process (treatment first with hydrochloric acid and then the action of aqueous vapour) is more efficacious.

While by this mode of treatment the oil issues from the chlorophyll-grains, the latter appear in all plants as if differentiated into a denser and a softer mass, and acquire a sponge-like aspect. The places of the soft substance which form the meshes of a net of which the denser substance consists, soon appear as true cavities from which the oil has escaped. In this way the whole grain finally appears to be *perforated like a sieve*, producing nearly the characteristic impression of a regular sieve-plate; or in those cases in which the sieve-like perforation does not appear very sharply, it shows a spongy-porous texture which reminds one of the differentiations of substance which occur in many states of cell-nuclei.

The constancy and uniformity with which this spongy-porous structure is displayed by careful treatment in all chlorophyll-bodies proves it to be their normal structure. The solid constituents form the framework; the oil and the chlorophyll-colouring-matter dissolved therein saturate it and fill up its pores.

It is impossible that this concordant structure and this definite form of the solid constituents should always occur uniformly in all chlorophyll-bodies, if this differentiation and distribution of the solid and fluid constituents were not normally expressed in the chlorophyll-grain. It is only complete saturation with oil that causes the latter to appear homogeneous in the normal state; and the solution of the chlorophyll-colouring-matter in the oil is at the same time the cause of the absorption-spectrum of the chlorophyll-bodies and of green leaves appearing displaced towards the red end, in opposition to the absorption-spectum of alcoholic and ethereal solutions of chlorophyll. For the oil and the hypochlorin, as is shown by every observation under the microscope, are powerful solvents of the chlorophyll-colouring-matter, and ,at the same time (like other solvents also) determine the tone of colour and the absorption-spectrum of the solution of chlorophyll; and upon this also depend the different colour-phenomena which may be observed under the microscope during the separation of the constituents of the chlorophyll-grain, in the escaping drops and the residuary solid framework.

During these separations, moreover, it is always easily perceived that the chlorophyll-colouring-matter is a simple and not a composite colouring-matter; but even here such phenomena may occur as in Fremy's so-called splitting of the chlorophyll-colouring-matter into its component parts. I have already exposed in detail what is erroneous in this notion in my first memoir* on chlorophyll. Here it will be sufficient, in order to exclude beforehand the same misconceptions of the colour-phenomena in the hypochlorin reaction, to call attention briefly to the fact that the chlorophyll-bodies in the tissues, like solutions of chlorophyll, when treated with hydrochloric acid, undergo a change of their tone of colour *before any separation* of the solid and fluid constituents, and acquire a golden-yellow tint. During the subsequent displacement of the hypochlorin and oil the greater part of the colouring-matter is carried away by these solvents, which thus, by its strong concentration in the separating drops, acquire the deep reddish-brown colour which renders the reaction so easily recognizable, whilst the solid frameworks remain more or less tinged with the grass-green or more bluish shades of the chlorophyll, and finally may appear but faintly tinted or even quite colourless.

II. *Formation of Hypochlorin in the Seedling.*

The demonstration here given of the general diffusion of hypochlorin and oil in the chlorophyll-bodies, necessarily raises the presumption of a close relation between these bodies, which are so rich in carbon, and the most important physiological function, the assimilatory activity of the green tissues.

Starch no longer appears to be the most widely diffused, predominant, or even sole formed product, rich in carbon, of the chlorophyll-apparatus; and this circumstance increases the doubts which *à priori* exist against the view that the starch-enclosures separated in the solid form constitute the primary product of assimilation. Unquestionably, *à priori*, the properties of a fluid or volatile oleaginous substance are much more in accordance with this; and even the extant observations upon the relative magnitudes of the exchange of gases during assimilation render it extremely probable that its primary product is not a hydrate of carbon, but a body poorer in oxygen. Moreover, a periodical escape of oleaginous drops from the chlorophyll-bodies into the sur-

* Monatsb. Berl. Akad. der Wiss., October 1874.

rounding protoplasm may in many cases be directly observed.

In this connexion the hypochlorin is especially worthy of notice, not only because it is never wanting in the fundamental substance of the chlorophyll-bodies, so far as these (as I shall show immediately) have been produced in the light, but also because it is apparently the only known substance which the seedling of the Angiospermia is unable to form from its reserve-materials without light. I have made a series of extended investigations on phanerogamous plants germinating in the dark in order to test whether a direct influence of light upon the formation of hypochlorin manifests itself.

I reared the seedlings from seeds in the dark until their reserve-materials were completely exhausted, and thus obtained the noteworthy result that the yellow etiolated seedlings at no stage of their development furnish indications of hypochlorin by the hydrochloric-acid reaction. This applies to all Angiospermia without exception; and although when we have to do with traces of a body in extensive tissues the demonstration of a negative result is a troublesome and tedious affair, and I have therefore hitherto been able to investigate only a moderate number of etiolated seedlings (*Finsterkeimlinge*) of various species, I can nevertheless, from the concordant results that I have obtained, assert with perfect certainty that not the least trace of hypochlorin occurs in the seedlings so long as they are not exposed to the light. This body originates in them only under the influence of light, after a longer or shorter action of the light upon the tissues which become green, and indeed at any age at which the etiolated young plant is exposed to the light, provided it is still capable of development. The rapidity of the viridescence of etiolated seedlings in the light depends, as is well known, upon the temperature and the intensity of light; and therefore, if one does not wish to employ artificial illumination and warmth, it is not a matter of indifference in what months the experiments are made. This applies also to the formation of hypochlorin in them.

I made my experiments in the summer months of July and August, with an average temperature of about 20°–23° C. (68°–73°·4 F.) in the place where they were carried on. At this high temperature of the air the etiolated seedlings become distinctly green in two or three hours when exposed to bright daylight, and in from 6 to 8 or, at the utmost, 10 hours they become quite a strong or even deep green.

On the investigation of the green tissues with hydrochloric

acid it then seemed that the formation of chlorophyll apparently long precedes that of the hypochlorin. To give an idea of the course of the phenomenon, I here append a few comparative data for etiolated seedlings of peas, hemp, cucumbers, and flax.

Etiolated seedlings 8–13 days old of these species of a *deep yellow* colour (the cotyledons of the cucumbers, as is often the case with these plants in spite of their having lived in the dark, having already a suspicion of green) show no trace of hypochlorin in their tissues when tested with hydrochloric acid.

Etiolated seedlings 8 days old, of the same plants and the same sowing, are rendered distinctly or even dark green by six hours exposure to light; but their tissues show no trace of hypochlorin.

Etiolated seedlings 8 days old, of the same sowing, exposed for 13–16 hours uninterruptedly to clear diffused daylight, behave similarly, although already dark green. No hypochlorin is yet to be found in their tissues.

Etiolated seedlings 8 days old, of the same sowing, placed in the light for 19–20 hours, show the first traces of hypochlorin, although on the whole sparingly.

Etiolated seedlings 8 days old, of the same sowing, exposed for 30–31 hours to full daylight, are full of hypochlorin. The plumules of the peas and hemp, the green tissue of the cotyledons of the cucumbers and flax, even the young scarcely coloured cells of their viridescent tissue, are now rich in hypochlorin.

From these investigations, therefore, it undoubtedly appears that in the Angiosperms the hypochlorin originates under the influence of light, and at the same time that it only becomes perceptible in them at a later period than the chlorophyll-colouring-matter.

There is undeniably a relation between the two substances. Does one of them proceed from the other? I cannot here go into this question, but will only point out that, from these experiments, as well as from the previous demonstration of its volatilization *without* destruction of the colouring-matter, the independent existence of the hypochlorin side by side with the chlorophyll-colouring-matter in the plant may be deduced with certainty. The green tissues, although they already contain the colouring-matter, nevertheless, when treated with hydrochloric acid, show no hypochlorin when they have not been exposed to the light for a considerable time. The hypochlorin therefore cannot originate only in the preparation from the colouring-matter, but must exist in the

plant together with the latter. This becomes still more distinct, and the connexion of assimilation with the formation of hypochlorin is rendered still clearer, when the above-described experiments on seedlings are slightly varied.

The viridescence of plants takes place, according to present notions, under a less intensity of light than assimilation. In half-obscurity, *i. e.* in strongly darkened places, therefore, seedlings become perfectly green, without, however, being able to keep themselves alive. They perish, not much later than when they vegetate in complete darkness. Although I regard the assumption that assimilation is entirely suppressed under small intensities of light as an error, the process is undoubtedly prejudiced; and therefore the accumulation of products rich in carbon, which the seedling requires after the consumption of its reserve-materials, is impossible under light of small intensity.

I have accordingly repeated the experiments on the production of hypochlorin with seedlings which I reared, not in the dark, but from the time of their germination in *half-obscurity.* In these, even in such as had lived from eight to fourteen days in half-obscurity, I likewise found no trace of hypochlorin, although the cotyledons, plumules, and primordial leaves of these little plants were well developed, and, especially, although these organs were as deeply and perfectly greened in the half-obscurity as is the case in seedlings which have been able to develop themselves quite freely and in full light for several days.

As a matter of course the result depends upon the light under which the plants grow; for even under moderate daylight in the place of experiment hypochlorin is present in the viridescent seedlings, and its quantity visibly increases with the increase of the light.

It is nevertheless not difficult to rear *beautifully green* seedlings without any trace of hypochlorin. This may be done, for example, by growing the plants in the experimental room at a great distance from the window and under bell-glasses covered with grey paper.

The just-demonstrated dependence of the formation of hypochlorin upon the influence of light would not *per se* prove a *direct* close relation to assimilation, but only indicate (as in the case of starch, fat, cellulose, and sugar) that it belongs to the series of those materials the storing up of which, as nearer or more remote products of assimilation, must necessarily be dependent upon the accumulation of carbon in the plant caused by light. This would certainly be the case if, in the instance of hypochlorin, as with the above-mentioned sub-

stances, we had to do only with an increase of the existing quantity in the light. But this is not the case; not merely have we to do here with an *increase of the existing quantity*, but hypochlorin, out of the whole series of materials which can come under consideration here, and especially of those which demonstrably occur together with it in the chlorophyll-apparatus, is the only one which cannot without light form itself in the seedling from the reserve-materials. Starch, oil, cellulose, and sugar, as is well known, reciprocally proceed from one another in the exchange of materials in the etiolated seedling, even without light. The green modification of the chlorophyll-colouring-matter alone has, in most Angiosperms, this property, in common with hypochlorin, of being unable to originate without light from the reserve-materials of the seedling; and this agreement of the two substances in such a decisive physiological point is certainly a noteworthy indication of common relations to the processes of assimilation, and of a direct interdependency.

The striking analogy shown by chlorophyll and hypochlorin in their relation to light in the Angiosperms extends very remarkably to the exceptional conditions of chlorophyll-formation in the Gymnosperms. As the Gymnosperms are the only Phanerogams whose seedlings can, in some unexplained fashion, form chlorophyll-colouring-matter in the dark, so also, singularly, the Gymnosperms are also the only ones in whose seedlings hypochlorin makes its appearance even in darkness.

I have paid particular attention to this peculiarity of the seedlings of Gymnosperms, and tested it in a geat number of comparative investigations on seedlings of *Pinus picea*, *montana*, *maritima*, and *Larix* grown in the dark.

Without going into a detailed description of the results and of the relation of the quantity of hypochlorin present to the age of the seedlings examined, I may here sum up the general result of this series of investigations as follows:—In the Coniferæ just mentioned hypochlorin occurs even in seedlings grown in the dark; and it may be indubitably ascertained that the viridescence of these seedlings in the dark precedes the presence of hypochlorin in them.

It is true that frequently, especially in *Pinus picea* and *montana*, there are scarcely any traces of hypochlorin in the viridescent cotyledons even in seedlings several weeks old (almost as late as the third week of germination); but, on the other hand, other examples of the same species already show noteworthy quantities of it; and if the little plants grow older in the dark, say about four or five weeks, it may be easily

detected in every cell of the green tissue, especially in *Pinus maritima.*

From the facts here communicated I believe that I have, in the first place, established anatomically and microchemically the individuality of the hypochlorin in the chlorophyll-bodies, and proved the necessity of light for its formation in the Angiosperms. With regard to the physiological relations of chlorophyll to hypochlorin I have already given some intimations, and expressed the opinion that chlorophyll, by means of its absorption of light, protects the hypochlorin from combustion in intense light. Upon the presumable genetic relations of chlorophyll to hypochlorin my investigations are not yet completed.

XXXII.—*On a Collection of Lepidoptera from Madagascar, with Descriptions of new Genera and Species.* By Arthur G. Butler, F.L.S., F.Z.S., &c.

The following species have been selected from a large collection made at Fianarantsoa by the Rev. W. Deans Cowan.

Rhopalocera.

Nymphalidæ.

Satyrinæ.

1. *Gnophodes betsimena* ♀.

Cyllo betsimena, Boisduval, Faune Mad. p. 58. n. 1 (1833).

A fine specimen.

In my Catalogue of Fabrician Lepidoptera I erroneously sunk this species as a synonym of *G. pythia*; now that we possess both I find the Madagascar species much nearer to the *G. parmeno* of Trimen from Natal, which is of the same size and form, but instead of a broad oblique white belt on the primaries has a rather narrow angulated ochreous one. As the *G. parmeno* of Trimen is not identical with the West-African form, I propose to call it *G. diversa.*

2. *Pseudonympha subsimilis.*

Pseudonympha subsimilis, Butler, Ann. & Mag. Nat. Hist. ser. 5, vol. iv. p. 228. n. 3 (1879).

The type was also taken at Fianarantsoa.